Establishment and Characterization of Methylmercury-resistant PC12 Cell Line

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Methylmercury (MeHg)-resistant sublines of rat pheochromocytoma (PC12) cells were isolated by repeated exposure to stepwise increased concentrations of MeHg. One of the sublines (PC12/TM) showed an 8- to 10-fold increase in resistance to MeHg compared with parent PC12 cells on the basis of the concentration required for 50% inhibition (IC_{50}) of growth. PC12/TM cells accumulated smaller amounts of MeHg than parent PC12 cells. This reduction in MeHg accumulation in PC12/TM cells resulted from slow uptake and rapid efflux. The intracellular glutathione (GSH) level in PC12/TM cells was four times higher than that of PC12 cells. Pretreatment of PC12/TM cells with buthionine sulfoximine, which decreased the GSH level to that of the parent PC12 cells, increased the sensitivity of PC12/TM cells to MeHg. A close correlation between the MeHg accumulation and MeHg sensitivity was found among seven sublines of PC12 cells and parent PC12 cell line. The GSH level in PC12 sublines was also correlated with their sensitivity to MeHg. — Environ Health Perspect 102(Suppl 3):313–315 (1994).

Key words: methylmercury, PC12 cell line, methylmercury resistant, methylmercury accumulation, methylmercury efflux, glutathione, buthionine sulfoximine

Introduction

MeHg poisoning is characterized by selective damage to neuronal cells (*1,2*). Numerous studies have been reported at the cellular level on the biochemical lesions caused by MeHg but less information is available concerning special susceptibility of neuronal cells compared with other cell types (*3,4*). The results obtained by using cell variants with various grades of resistance to some drugs and metals suggest that differences in susceptibility of the cells to some drugs and metals were related to differences in cellular accumulation of these agents (*5,6*). In the case of cadmium, enhanced resistance to this metal by human cell lines also correlated with reduction of cadmium accumulation (*6*). With respect to MeHg, however, no mammalian cells with different sensitivities to MeHg have been described.

This study were undertaken with the aim to establish a mammalian cell line with varying levels of sensitivity to MeHg and to clarify the factors which alter the sensitivity of mammalian cells to MeHg. MeHg-resistant sublines of rat pheochromocytoma (PC12) cells were established in this study (*7*). Furthermore, by using these sublines it was suggested that the MeHg accumulation and intracellular GSH level play important roles in the susceptibility of PC12 and its sublines to MeHg.

Materials and Methods

The PC12 cells were maintained in a growth medium consisting of D-MEM supplemented with 10% fetal bovine serum, 5% equine serum, and 50 μg/ml gentamicin at 37°C in a humidified atmosphere containing 5% CO_2.

The initial induction of resistance was achieved by exposure of PC12 cells to 3 μM MeHg (CH_3HgCl) for 3 days. The surviving population of parent cells was maintained for 2 months in the medium without MeHg, then the cells were further treated with 5 μM MeHg for 4 days. The surviving cells were maintained in MeHg free medium for 2 weeks and cloned (*7*). One of the sublines (PV12/TM) was chosen for further characterization.

Cytotoxicity of MeHg was measured by growth inhibition or colony-forming assay (*7*). Accumulation of MeHg in the cells was measured after the incubation with growth medium containing serum and [^{203}Hg]-MeHg at 37°C for 1 to 60 min. Efflux of MeHg from the cells preloaded with 5 μM [^{203}Hg]-MeHg for 60 min was measured after incubating further for 1 to 60 min with MeHg-free growth medium containing serum. Intracellular GSH level was determined by high performance liquid chromatography using ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate as a fluorogenic reagent according to the method of Toyo'oka and Imai (*8*).

Results and Discussion

The MeHg-resistant PC12/TM cells are similar in size and morphology to the parental PC12 cells. Intracellular GSH levels of PC12 and PC12/TM cells were

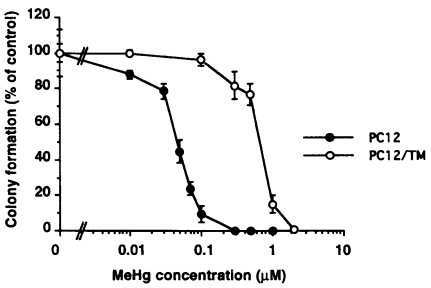

Figure 1. Sensitivity of PC12($_\Sigma$) and PC12/TM($_0$) cells to MeHg as determined by colony-forming assay. Colony-forming activity after incubation in the presence of MeHg for 10 days was measured. Each point is the mean of 3 observations and the vertical bars represent the standard deviations.

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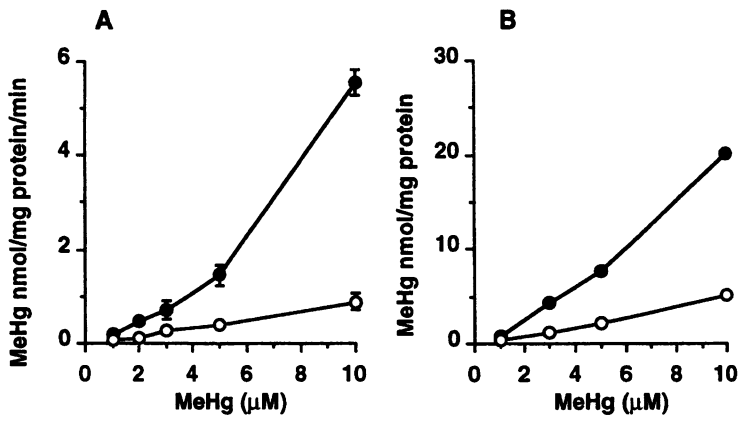

Figure 2. Dose dependency of MeHg uptake for initial 1 min (*A*) and 60 min (*B*) in PC12 (●) and PC12/TM(○) cells. Cells were incubated for 1 min (*A*) and 60 min (*B*) in the presence of various concentrations of MeHg. Each point is the mean of three or four observations and the vertical bars represent the standard deviations. In Figure 2*B* the standard deviation is less than 0.28 nmole/mg protein at all points.

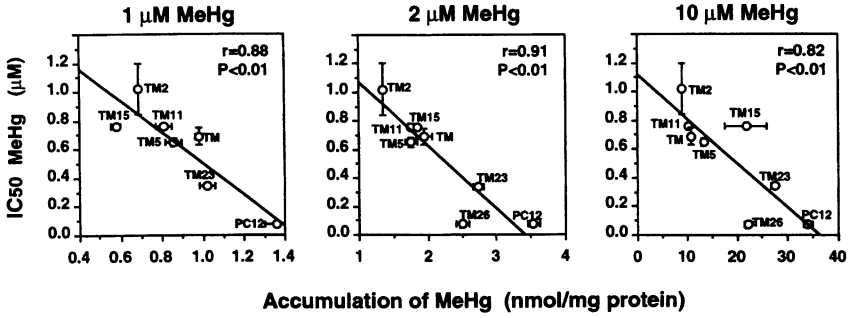

Figure 3. Correlation between sensitivity to MeHg (IC_{50}) and accumulation of MeHg in sublines of PC12 cells. The IC_{50} was measured by colony-forming assay in the presence of MeHg for 10 days. Accumulation of MeHg was determined by incubating the cells with medium containing [^{203}Hg]-MeHg (1, 2, and 10 μM) for 60 min at 37°C in the presence of serum. PC12/TM sublines are labeled TM2, TM5, TM11, TM15, TM23, TM26, and PC12.

8.7±0.35 and 36.7±1.95 nmole/mg protein, respectively. The resistance to MeHg and the high level of intracellular GSH in PC12/TM cells have been maintained for more than one year under culture conditions without MeHg.

PC12/TM cells showed about an 8-fold increase in resistance to MeHg compared with the parent PC12 cells on the basis of the concentration required for 50% inhibition (IC_{50}) of growth under the condition of the exposure to MeHg for 24 hr. On chronic exposure to MeHg for 10 days, the IC_{50} value for PC12/TM cells was about 10-fold greater than that of parent PC12 cells (Figure 1).

Incorporation of MeHg into PC12 cells occurred more rapidly than into PC12/TM cells, and MeHg attained a higher level in the PC12 cells than in PC12/TM cells after 60 min. Accumulation of MeHg in PC12/TM cells was dose-dependent in the concentration ranges of 1 to 10 μM but only reached approximately 1/2 to 1/4 of that in PC12 cells (Figure 2*B*). Uptake of MeHg in a short period (1 min) was also significantly lower in the MeHg-resistant PC12/TM cells than in the parent PC12 cells (Figure 2*A*).

Efflux of MeHg from the cells preloaded with 5 μM MeHg for 60 min was linear for approximately 5 min in both cell lines, thereafter approaching a plateau level. The efflux rates calculated from the slopes over the first 5 min were 0.030 and 0.086 nmole/mg protein/min in PC12 and PC12/TM cells, respectively. Thus, about 80% of MeHg was released out of the PC12/TM cells during incubation for 60 min, while less than 20% of MeHg was lost from the parent PC12 cells. These results indicate that the reduced MeHg accumulation in PC12/TM cells is due to their slow uptake and the rapid efflux of MeHg.

The intracellular GSH level in PC12/TM cells was four times higher than that of PC12 cells. Pretreatment of PC12/TM cells with buthionine sulfoximine (BSO), which reduced the GSH level to that of the parent PC12 cells, increased the sensitivity of PC12/TM cells to MeHg, as shown by the decreasing IC_{50} value from 11 to 5 μM. Since the accumulation of MeHg in PC12/TM cells after treatment with BSO remained unchanged, the intracellular GSH may prevent cell damage by interrupting MeHg binding to the target molecules in the cells.

The relationship between sensitivity to MeHg and MeHg accumulation or intracellular GSH levels was investigated by using seven sublines (PC12/TM2, PC12/TM5, PC12/TM11, PC12/TM15, PC12/TM23, PC12/TM26, and PC12/TM) of PC12 cells with different sensitivity to MeHg (*7*). MeHg accumulation for 60 min at 1, 2, and 10 μM MeHg in the medium was well correlated with sensitivity to MeHg (Figure 3): the least squares linear correlation was found to be significant at 1 μM ($p<0.01$, $r=0.88$), 2 μM ($p<0.01$, $r=0.91$) and 10 μM ($p<0.01$, $r=0.82$). The GSH levels in these seven sublines of PC12 cells were also correlated with the sensitivity to MeHg ($p<0.05$, $r=0.72$).

From these results we concluded that the MeHg accumulation and intracellular GSH level might play important roles in determining the sensitivity of PC12 and its sublines to MeHg.

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